JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Simultaneous Estimation of Sacubitril and Valsartan in Synthetic Mixture by RP-HPLC Method

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ABSTRACT:

A simple, accurate, rapid and precise isocratic reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of Sacubitril and Valsartan in synthetic mixture. The chromatographic separation was carried out on C18 (250*4.6 mm, 5µm) column with a mixture of Acetonitrile: methanol: water, pH 3 adjusted with ortho-phosphoric acid (30:50:20, %v/v) as mobile phase;at a flow rate of 1.0 ml/min. UV detection was performed at 267 nm. The retention times were 2.464 and 3.264 min. for Sacubitril and Valsartan respectively. Calibration plots were linear over the concentration range 50-250 µg/ml for Sacubitril and 50-250 µg/ml Valsartan. The method was validated for system suitability, accuracy, precision, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Sacubitril and Valsartan.

KEYWORDS: Sacubitril; Valsartan; RP-HPLC, Acetonitrile: methanol, simultaneous determination.

Article history:

Received 01 April 2016
Revised 14 April 2016
Accepted 16 April 2016
Available online 20April 2016

Citation:

Patel K. H. , Luhar S. V., Narkhede S. B. Simultaneous Estimation of Sacubitril and Valsartan in Synthetic Mixture by RP-HPLC Method. **J Pharm SciBioscientific Res**. 2016, 6(3):262-269

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1. INTRODUCTION:[1-3, 8-14]

Liquid chromatography is the most widely used analytical tool in the pharmaceutical industry and reversed-phase is the most frequently used mode. During the drug development process, liquid chromatographic methods are used to determine the quality of the drug substance (active pharmaceutical ingredient) and drug product.Sacubitrilis chemically (S)-5-[(4-phenylphenyl)methyl] pyrrolidin-2-one belongs to the class of neprilysin inhibitor, used as anti-hypertensive. Molecular Formula –$C_{17}H_{17}NO$, Molecular Weight – 251.32 g/mol, Solubility - Slightly soluble in water, sparingly soluble in dehydrated alcohol, freely soluble in methanol. Valsartan is chemically (2S)-3-methyl-2-(N-{[2'-(2H-1,2,3,4-tetrazole-5-yl)biphenyl-4-yl]methyl} pentanamido) butanoic acid.Valsartan is potent Angiotensin II receptor blocker. Itis mainly used as anti-hypertensive drug.Valsartan isofficial in IP and USP. The (S)enantiomer is essentially used. Molecular Formula –$C_{24}H_{29}N_5O_3$, Molecular weight- 435.5 g/mol and Soluble in Acetonitrile, practicallyinsoluble in water also soluble in methanol. The aim of the present study was todevelop accurate, precise and selective reverse phase HPLCassay procedure for the analysis of Sacubitril and Valsartan in synthetic mixture. The validation ofproposed method is done according to the ICH guidelinevalidation was done according to ICH guidelines.

From the literature survey it was found that many methods are available for determination of Valsartan individually and few methods in combination with other drugs. However, no HPLC method has been reported for simultaneous determination ofSacubitril and Valsartan in combination. In the proposed study an attempt will be made to develop a HPLC method for simultaneous estimation of Sacubitril and Valsartan. Pharmaceutical grade of Sacubitril and Valsartan were kindly supplied as gift samples by Manus Akketeva, Ahmadabad, India and Lupin Ltd respectively, certified to contain > 99% (w/w) on dried basis. All chemicals and reagents used were of HPLC grade and were purchased fromChemicals, Ran Kem,India.

2. MATERIALS AND METHODS

2.1 METHOD DEVELOPMENT

2.1.1 Equipment:

Chromatographic separation was performed on HPLC system consist of model Shimadzu having UV-Vin detector and injector with 10µl loop volume. LC solution software was applied for data collecting and processing.

2.1.2 Reagents and chemicals: Acetonitrile and methanol of HPLC grade were procured from RanKem lab ltd. Sacubitril and Valsartan standards were received as gift samples from Manus Akketeva and Lupin Ltd, India, respectively

2.1.3 Selection of detection wavelength:

The standard solution of Sacubitril (10 µg/ml) and Valsartan (10 µg/ml) in methanol was individually scanned over the range of 200 nm-400 nm. Its overlay graph showed that both the drug absorb at 267 nm (As show in figure- 3). So, the wavelength selected for the determination of Sacubitril and Valsartan was 267 nm.

2.1.4 HPLC Conditions:

A SheisedoC$_{18}$ (250*4.6 mm, 5µm) column was used as the stationary phase. A mixture of Acetonitrile, methanol and water in the ratio of (30 : 50: 20 %v/v) was used as a mobile phase and pH 3.0 adjusted with Ortho phosphoric acid. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 267nm.The injection volumes of sample and standard were 10µl.

2.1.5 Standard solutions:

A stock solution containing 1000µg/ml of Sacubitril and Valsartan were prepared separately by dissolving in methanol. A working standard solution containing 50-250 µg/ml and 50-250 µg/ml of Sacubitril and Valsartan were prepared from the above stock solution. All the stock solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

2.2. ASSAY OF TABLET FORMULATION:

To determine the content of Sacubitril and Valsartan simultaneously in conventional tablet (ENTRESTO, label claim 24 mg Sacubitril 26 mg Valsartan); twenty tablets were accuratelyweighed, average weight was determined and grounded to fine powder.

A quantity of powder equivalent to 24 mg Sacubitril and 26 mg Valsartan was transferred into 100 ml volumetric flask containing 50 ml Methanol, sonicated for 10 min. and diluted to mark with same solvent to obtain 240 µg/ml Sacubitril and 260 µg/ml Valsartan.

The resulting solution was filtered using Whatman filter paper. From the above solution 3 ml was transferred into 10 ml volumetric flask and diluted to mark with same solvent. So, Resultant solution was found to contain 72 µg/ml Sacubitril and 78 µg/ml Valsartan.

This Test solution was injected and chromatogram was recorded for the same The amount of drugs were calculated and the results are given in Table 3.

2.3 METHOD VALIDATION [7]

The developed method was validated as per ICH guidelines for its System suitability, linearity, accuracy, precision,robustness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in Table.

2.3.1 System suitability

System suitability and chromatographic parameters were validated such as resolution, theoretical plates, and tailing factorwere calculated.

2.3.2 Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Sacubitril and Valsartan at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance Vs concentration of the drug. The responses were found to be linear in the range 50-250 µg/ml and 50-250 µg/ml for Sacubitril and Valsartan.

2.3.3Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 4 different concentration levels (0%, 80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

2.3.4 Precision

a) Repeatability

Standard solutions of 100, 150, 200 µg/ml Sacubitril and 100, 150, 200 µg/ml Valsartan were prepared and Chromatogram were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

b) Intraday precision

Mixed solutions containing 100, 150, 200 µg/ml of Sacubitril and Valsartan were analyzed three times on the same day and % R.S.D was calculated.

c) Interday precision

Mixed solutions containing 100, 150, 200 µg/ml of Sacubitril and Valsartan were analyzed three times on different days and % R.S.D. was calculated.

2.3.5 Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines.

2.3.6. Robustness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of both drugswas noted. The factors selected were flow rate, pH of the mobile phase and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters.

3. RESULTS & DISSCUSION

3.1. System suitability

System suitability and chromatographic parameters were validated such as resolution, theoretical plates, and tailing factor was calculated. The result is given in Table 4

3.2. Linearity

The calibration curve showed (Fig.4, 5, 6) good linearity in the range of 50-250 µg/ml, for Sacubitril with correlation coefficient (r^2) of 0.9982 and 50-250 µg/ml for Valsartan with correlation coefficient (r^2) of 0.9972. Results are given in Table 5.

3.3 Precision

Intraday precision was carried out using test samples prepared and analyzed on the same day. Interday precision was assessed by analysis of the same solutions on consecutive days. The low % RSD values below 2 indicate that the method is precise. Repeatability also performed. The results are given in table 6, 7, 8.

3.4 Accuracy

At each concentration, sample was injected thrice to check repeatability and from the %RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.25-100.90% for the Sacubitril and 99.59-101.05% for valsartan at three different concentrations 80%, 100%, 120%. The results are given in Table 9, 10.

3.5. Robustness

Small deliberate changes in chromatographic conditions such as change in mobile phase ratio (± 2 %), change in pH (±2 units) and flow rate (± 2 units) were studied to determine the robustness of the method. The results were in factor of (% RSD< 2%) the developed RP-HPLC method for the analysis of Sacubitril and Valsartan. The results are given in Table 11, 12**.**

3.6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were found to be 19.28 µg/ml and 58.45 µg/ml for Sacubitril and 23.85 µg/ml and 72.27

µg/ml for Valsartan estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration. The result is given in Table 13.

3.7. DISCUSSION

A simple, accurate and precise RP-HPLC method for the simultaneous estimation of Sacubitril and Valsartan in synthetic mixture has been developed and validated.

RP-HPLC method was found to be linear over the range of 50-250 µg/ml for Sacubitril and Valsartan.

Separation of drugs was carried out using Acetonitrile: Methanol: water (pH-3.0) (%V/V) (30 : 50: 20:) mobile phase at 5 min. run time and 267 nm. The $R_t$value for Sacubitril and Valsartan was found to be 2.464 and 3.264 min respectively.

The method has been validated for linearity, accuracy and precision, L.O.D., L.O.Q. and system suitability according to ICH guideline.

4. CONCLUSION

- A simple, rapid, accurate and precise RP-HPLC method was developed and validated for Simultaneous estimation of Sacubitril and Valsartan in Synthetic mixture.
- For RP-HPLC method linearity range was found in range of 50-250 µg/ml for Sacubitril and Valsartan.
- Limit of detection and Limit of Quantification was found to be 19.28 µg/ml and 58.45 µg/ml for Sacubitril and 23.85 µg/ml and 72.27 µg/ml forValsartan.
- % RSD for intraday ≤ 2 and interday precision was found to be ≥ 2.
- % Recovery greater than 98 but less than 102 for this method shows that the method is accurate and free from the interference of excipients used in formulation.
- So, the developed method can be used for routine analysis and quality control test for Sacubitril and Valsartan.

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6. List of Figures & Tables

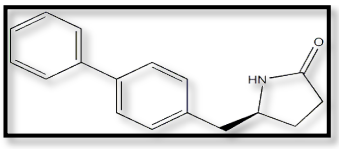

Figure 1- Chemical structure of Sacubitril

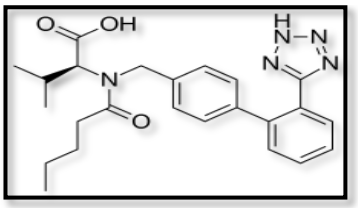

Figure 2 - Chemical structure of Valsartan.

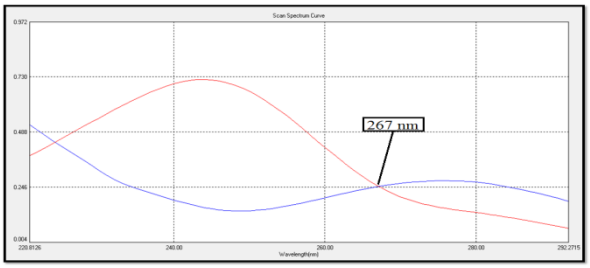

Figure3 : Selection of detection wavelength for HPLC

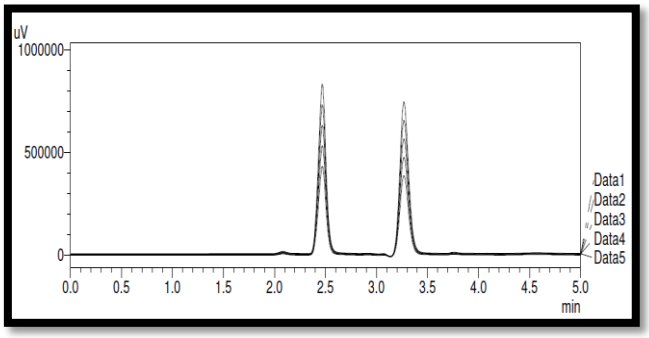

Figure 4: Chromatogram of Sacubitril and Valsartan

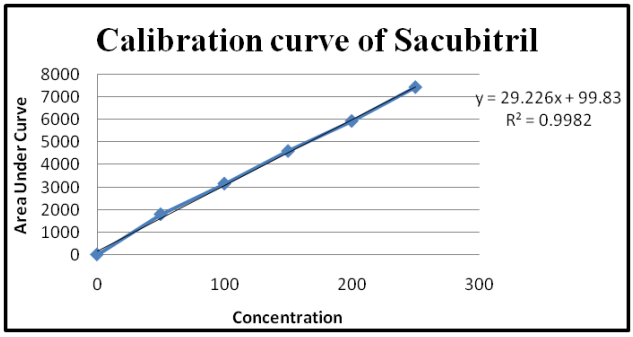

Figure 5 : Calibration curve of Sacubitril

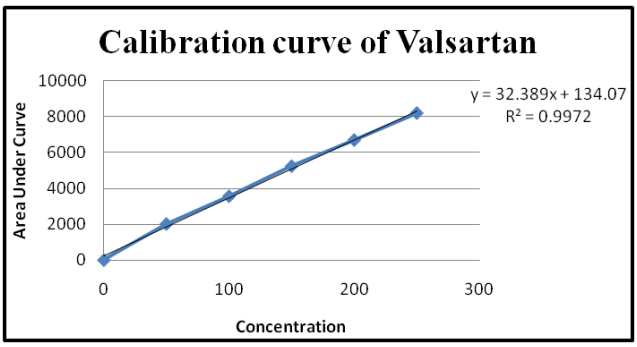

Figure 6: Calibration curve of Valsartan

Table 1 : Chromatographic condition

Parameters	Specifications
Column	Sheisedo C18 (250mm * 4.6mm, 5 µm)
Mobile phase	Acetonitrile : Methanol : Water (pH-3.0) (30:50:20 %V/V)
Flow rate	1 ml/min
Run time	5 min
Detection wavelength	267 nm
Retention time	2.465 min for Sacubitril and 3.264 min for Valsartan

Table 2 : Calibration data of Sacubitril and Valsartan

Sacubitril		Valsartan	
Conc. (µg/ml)	**Mean Area ± SD**	**Conc. (µg/ml)**	**Mean Area ± SD**
0	0	0	0
50	1791.958 ± 6.641	50	2013.349 ± 6.257
100	3155.138 ± 6.649	100	3560.336 ± 2.856
150	4606.071 ± 5.317	150	5236.454 ± 6.129
200	5942.507 ± 8.806	200	6692.974 ± 4.329
250	7448.580 ± 7.743	250	8139.216 ± 5.160

Table 3 : Assay result of Marketed formulation

Drug	Actual conc. Of Drug (µg/ml)	Amt. OfDrug Found (µg/ml)	% of Drug found	Avg. Of % Drug found	SD	%RSD
	72	71.98	99.97			
Sacubitril	72	72.05	100.06	99.94	0.1334	0.1335
	72	71.86	99.80			
	78	77.99	99.98			
Valsartan	78	79.08	101.38	100.38	0.8676	0.8643
	78	77.84	99.79			

Table 4 :System Suitability Parameters for Sacubitril and Valsartan

Sr. No.	System suitability parameter	Sacubitril	Valsartan
1	Conc. (µg/ml)	50	50
2	Retention time (min)	2.464	3.264
3	Resolution (R)	30427	
4	Theoretical plate number (N)	6608.475	4094.916
5	Tailing factor (T)	1.515	1.477

Table 5 : Linearity

Conc. (µg/ml)	Sacubitril		Valsartan	
	Area. Mean ± S.D	% RSD	Area. Mean ± S.D	% RSD
0	0	0	0	0
50	1794.98 ± 1.6172	0.09	2012.906 ± 1.7754	0.0882
100	3154.602 ± 1.2261	0.0388	3563.406 ± 2.0082	0.0563
150	4604.002 ± 2.6314	0.0571	5236.885 ± 1.7437	0.0332
200	5972.218 ± 2.1563	0.0362	6694.349 ± 2.0907	0.0312
250	7449.01± 1.5947	0.0214	8194.533 ± 2.0426	0.0249

Table 6 : Repeatability data for Sacubitril and Valsartan

Conc. (µg/ml)	Sacubitril		Valsartan	
	Mean Area ± S.D. (n = 3)	%R.S.D.	Mean Area ± S.D. (n = 3)	%R.S.D.
100	3163.728 ± 4.9288	0.1557	3557.011 ± 5.2491	0.1475
150	4605.182 ± 5.7308	0.1244	5243.716 ± 5.7858	0.1103
200	5943.360 ± 5.4325	0.0914	6695.234 ± 3.7027	0.0553

Table 7 : Intraday data for Sacubitril and Valsartan

Conc. (µg/ml)	Sacubitril		Valsartan	
	Mean Area ± S.D. (n = 3)	%R.S.D.	Mean Area ± S.D. (n = 3)	%R.S.D.
100	3158.791 ± 7.5724	0.2392	3561.054 ± 7.4041	0.2079
150	4613.182 ± 6.8473	0.1484	5239.335 ± 9.2765	0.177
200	5944.027 ± 8.4847	0.1427	6687.389 ± 11.8126	0.1766

Table 8 : Interday data for Valsartan

Conc. (µg/ml)	Sacubitril		Valsartan	
	Mean Area ± S.D. (n = 3)	%R.S.D.	Mean Area ± S.D. (n = 3)	%R.S.D.
100	3158.791 ± 7.5724	0.2392	3561.054 ± 7.4041	0.2079
150	4613.182 ± 6.8473	0.1484	5239.335 ± 9.2765	0.177
200	5944.027 ± 8.4847	0.1427	6687.389 ± 11.8126	0.1766

Table 9 : Accuracy data for Sacubitril

%Recovery	Target Conc.	Spiked Conc.	Final Conc.	Conc. Obtained	%Assay
0 %	100	0	100	99.25	99.25
	100	0	100	100.5	100.5
	100	0	100	99.39	99.39
	100	80	180	180.62	100.34
80 %	100	80	180	179.31	99.61
	100	80	180	179.72	99.84
	100	100	200	199.86	99.93
100 %	100	100	200	200.19	100.09
	100	100	200	200.36	100.18
	100	120	220	220.13	100.05
120%	100	120	220	219.86	99.93
	100	120	220	221.99	100.90

Table 10 : Accuracy data for Valsartan

%Recovery	Target Conc.	Spiked Conc.	Final Conc.	Conc. Obtained	%Assay
0 %	100	0	100	100.16	100.16
	100	0	100	100.23	100.23
	100	0	100	99.59	99.59
	100	80	180	179.81	99.89
80 %	100	80	180	181.89	101.05
	100	80	180	180.38	100.21
	100	100	200	200.08	100.04
100 %	100	100	200	199.93	99.96
	100	100	200	199.71	99.85
	100	120	220	220.26	100.11
120%	100	120	220	220.45	100.20
	100	120	220	219.68	99.85

Table 11 : Robustness data for Sacubitril

Sr. No.	Sacubitril (150 µg/ml)					
	pH		**Flow rate**		**Mobile phase**	
	(+0.2 Unit)	(-0.2 unit)	(+0.2 unit)	(-0.2 unit)	(+2.0 %)	(-2.0%)
1	4604.542	4528.946	4619.843	4539.231	4643.546	4543.287
2	4600.246	4519.754	4612.169	4533.561	4635.854	4550.842
3	4610.369	4521.695	4622.861	4543.215	4649.643	4536.102
SD	5.0807	4.8448	5.5123	4.8514	6.9098	7.3707
Mean	4605.052	4523.465	4618.291	4538.669	4643.014	4543.410
%RSD	0.1103	0.1071	0.1193	0.1068	0.1488	0.1622

Table 12: Robustness data for Valsartan

Sr.no.	Valsartan (150 µg/ml)					
	pH		**Flow rate**		**Mobile phase**	
	(+0.2 Unit)	**(-0.2 unit)**	**(+0.2 unit)**	**(-0.2 unit)**	**(+2.0%)**	**(-2.0%)**
1	5236.454	5145.289	5221.689	5156.896	5229.128	5134.543
2	5230.258	5139.423	5228.276	5144.286	5234.189	5139.286
3	5225.139	5149.964	5219.421	5158.456	5222.421	5128.943
SD	5.6660	5.2817	4.5996	7.7699	5.9031	5.1774
Mean	5230.617	5144.892	5223.128	5153.212	5228.593	5134.257
%RSD	0.1083	0.1026	0.0880	0.1507	0.1129	0.1008

Table 13 : L.O.D. and L.O.Q. data for Sacubitril and Valsartan

Parameter	Sacubitril	Valsartan
L.O.D.	11.2721	13.6619
L.O.Q.	34.1579	41.3991

Table14 : Summery of Validation parameter

Parameter	Sacubitril	Valsartan
Linearity range (µg/ml)	50-250	50-250
Correlation coefficient	0.9982	0.9972
Repetability (%R.S.D.)	0.0914-0.1557	0.0553-0.1475
Intraday precision (%R.S.D.)	0.1309-0.2011	0.0592-0.1475
Interday precision (%R.S.D.)	0.1427-0.2392	0.1766-0.2079
Mean % Recovery	99.25-100.90	99.59-101.05
Robustness (%R.S.D.)	0.1068-0.1622	0.0880-0.1507
LOD (µg/ml)	19.2889	23.850
LOQ (µg/ml)	58.4513	72.2745